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NextRAD phylogenomics, sanger sequencing and morphological data to establish three new species of New Guinea stream beetles

Michael Balke[1], Rawati Panjaitan[2], Suriani Surbakti[3], Helena Shaverdo[4], Lars Hendrich[5], Matthew H. Van Dam[6,7], Athena Lam[7]

1 *SNSB-Zoologische Staatssammlung München, Münchhausenstraße 21, D-81247 Munich, Germany and GeoBioCenter, Ludwig Maximilians University, Munich, Germany*
2 *Department of Biology, Faculty of Sciences and Mathematics, University of Papua (UNIPA), Manokwari, West Papua, Indonesia*
3 *Department of Biology, Universitas Cenderawasih, Waena, Papua, Indonesia*
4 *Naturhistorisches Museum Wien, Burgring 7, 1010 Vienna, Austria*
5 *SNSB-Zoologische Staatssammlung München, München, Germany*
6 *Entomology Department, Institute for Biodiversity Science and Sustainability, California Academy of Sciences, 55 Music Concourse Dr., San Francisco, CA 94118, USA*
7 *Center for Comparative Genomics, Institute for Biodiversity Science and Sustainability, California Academy of Sciences, 55 Music Concourse Dr., San Francisco, CA 94118, USA*

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Corresponding author: Michael Balke (balke.m@snsb.de)

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Abstract

We use molecular phylogenomic as well as morphological data to provide a taxonomic update on New Guinea endemic *Philaccolilus* diving beetles. In these lotic beetles, we find cryptic diversity that highlights the need for geographically denser sampling combined with the use of an intergrative taxonomic approach to unravel the true diversity and biogeography of these beetles. We describe three new species: *P. intania* **sp. nov.** from the northern Bird's Head Peninsula, *P. kirana* **sp. nov.** from the southern Bird's Head as well as *P. febrina* **sp. nov.** which is more widespread on the Bird's Head. *Philaccolilus ameliae weylandensis* is elevated to species rank, as *Philaccolilus weylandensis* **stat. nov.**

Key Words

Dytiscidae, *Philaccolilus*, new species, New Guinea mountain ranges, population genomics, morphology

Introduction

The diving beetle genus *Philaccolilus* Guignot, 1937 is endemic to the island of New Guinea. To date, thirteen species and one subspecies were recognized (Balke et al. 2018; Nilsson and Hájek 2022). These beetles are among the few New Guinea diving beetles with strict lotic habitat preference. They inhabit, specialized on the species level, lower order forest streams, a variety of different size mountain creeks and streams, and some even lowland rivers. In larger streams and rivers, their typical habitat is fast streaming water at the edge, on sandy bottom and with presence of plants and / or roots in the water. In lower order streams, the beetles can be observed swimming on the sandy / gravelly bottom of small pools underneath cascades. Most of these habitats do experience very heavy flooding during rainfalls, which leads to some degree of downstream drift, as inferred from finding single specimens of forest species along the margin of large rivers after flooding.

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The beetles usually show a characteristic and strongly contrasting yellow / orange and black dorsal color pattern (Figs 1–3), also seen in other stream inhabiting diving beetles (e.g. species in the genera *Platynectes* Régimbart, 1879, *Neptosternus* Sharp, 1882, *Philaccolus* Guignot, 1937, *Laccodytes* Régimbart, 1895, *Laccophilus* Leach, 1815) (see Balke et al. 2000).

To study the population structure and biogeography of *Philaccolilus* species, we have previously presented data from nextRAD sequencing. This is an approach to perform genotyping by sequencing and collect genomic data e.g. for population genomic questions (Lam et al. 2018). We discovered hidden diversity in the clades studied, with larger scale geographic structuring. At the same time, we find idiosyncratic patterns of population connectivity. On one hand, populations separated geographically by significant mountain and lowland regions show low degrees of genetic divergence. On the other

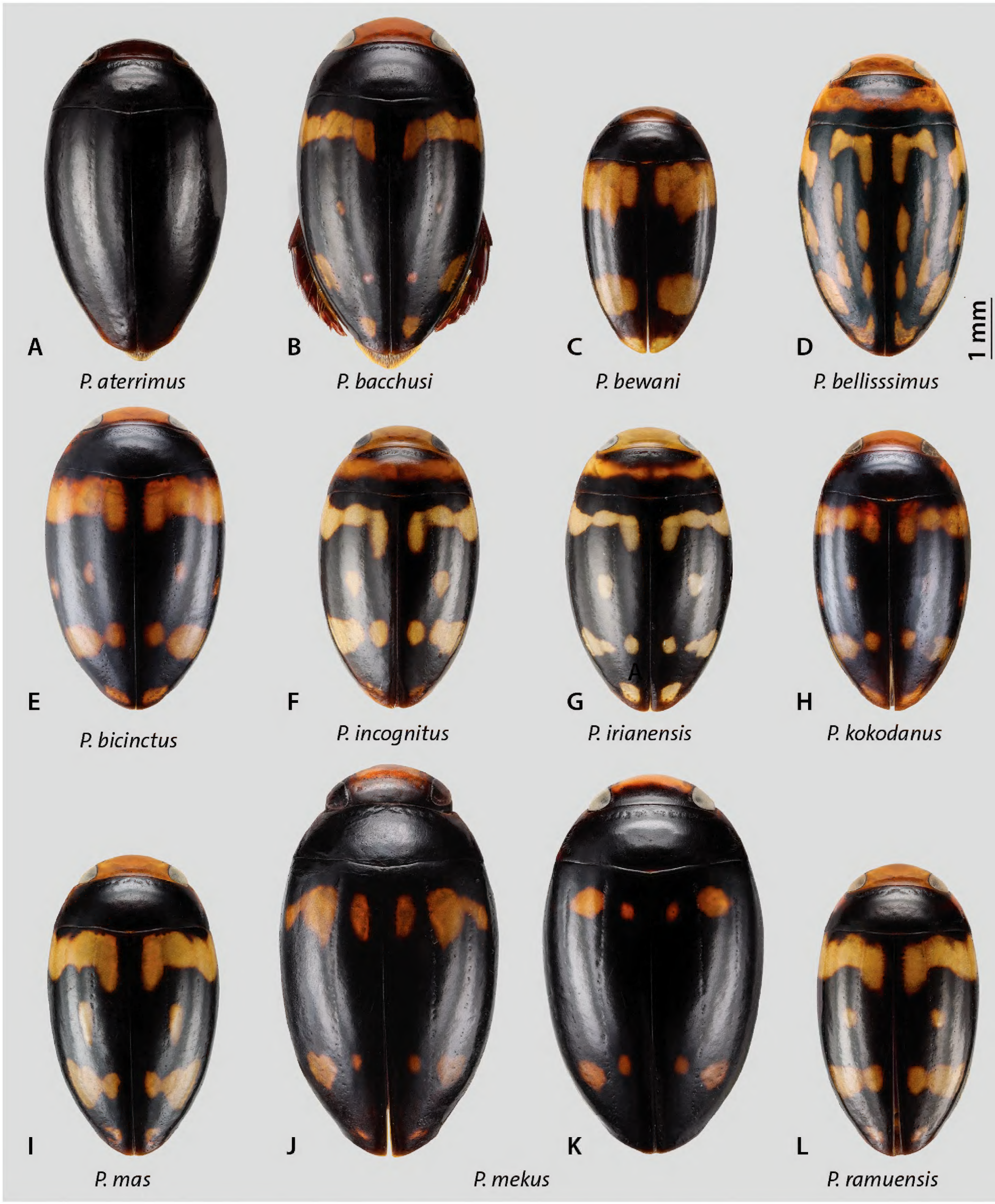

Figure 1. A–L. Dorsal habitus of *Philaccolilus* species, appendages mostly removed; brightness of paler spots digitally slightly enhanced for clarity, without altering the basic color tone.

hand, geographically closer populations of the same putative "morphological" species were genetically comparably divergent (Lam et al. 2018). Here, we provide a morphological evaluation of newly discovered lineages, also adding data from PCR based Sanger sequencing. We suggest three new species, and raise one previously suggested subspecies to species status.

This work is the continuation of our long-term engagement with the State University of Papua (UNIPA) and the Department of Biology, Universitas Cenderawasih, Waena, Papua, Indonesia (UNCEN) facilitate by reciprocal visit of staff and joint lectures and field training (see e.g. Balke et al. 2018; Cancian de Araujo et al. 2018).

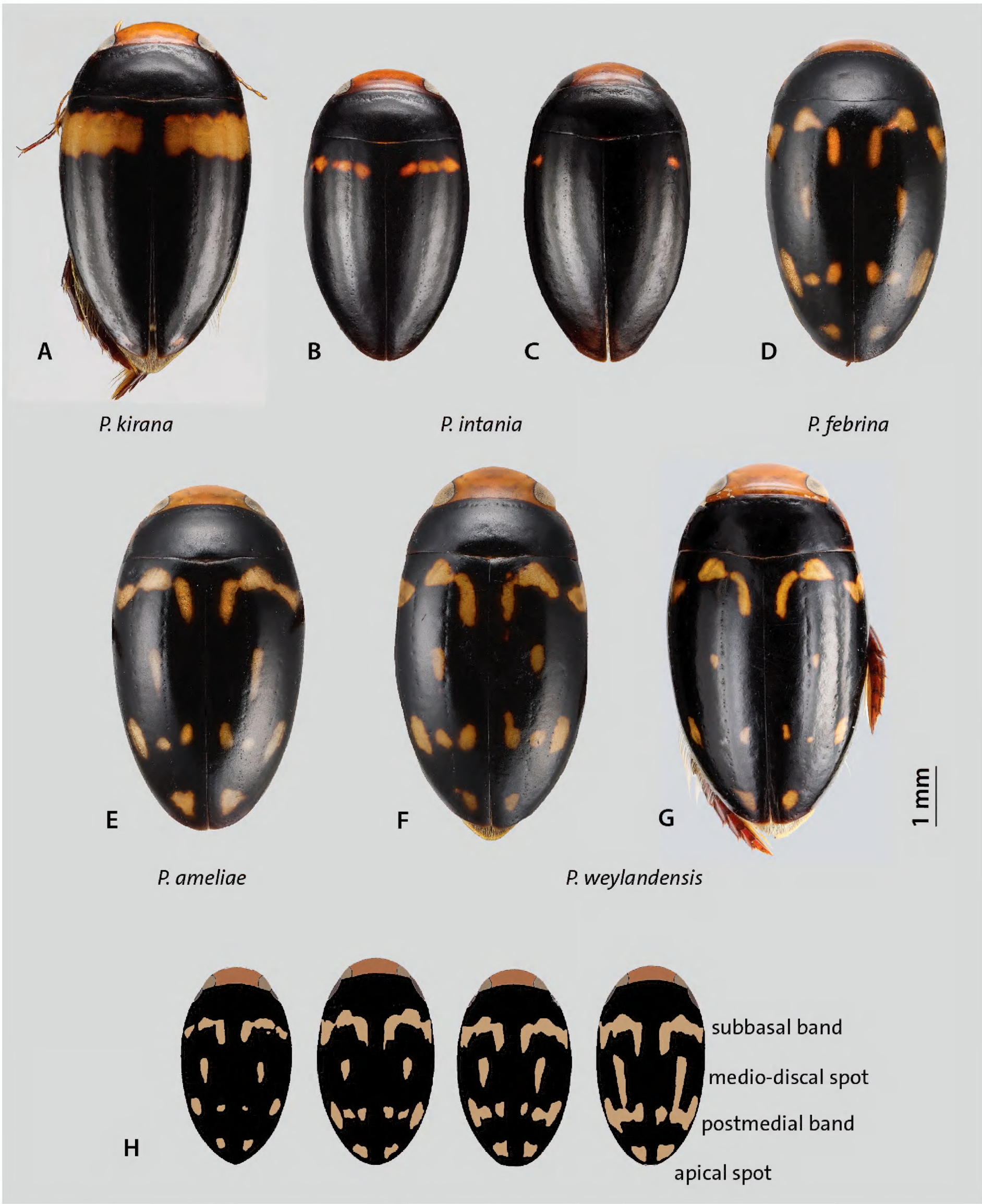

Figure 2. A–G. Dorsal habitus of *Philaccolilus* species in the *P. ameliae* complex, as well as *P. kirana* and *P. intania*, appendages mostly removed; brightness of some paler spots digitally slightly enhanced for clarity, without altering the basic color tone; **H.** schematic drawings of color pattern variation in *P. ameliae* species complex, with explanation of elytral pattern positions (modified from Balke et al. 2000).

Figure 3. Lateral habitus of *Philaccolilus* species; brightness of some paler spots digitally slightly enhanced for clarity, without altering the basic color tone. **A**. *P. kirana*; **B**. *P. intania*; **C**. *P. weylandensis* (Weyland Mts., basal lateral spot clearly reaching elytral margin); **D**. *P. weylandensis* (Digul River, basal lateral spot vaguely reaching elytral margin); **E**. *P. ameliae* (Waaf, basal lateral spot clearly isolated from elytral margin).

Materials and methods

The following acronyms are used in the text: NARI (Papua New Guinea National Insect Collection, Port Moresby, PNG); MZB (Museum Zoologicum Bogoriense, Cibinong, West Java, Indonesia), KSP (Koleksi Serangga Papua, at the Biology Department of Universitas Cenderawasih (UNCEN), Waena, Papua, Indonesia) and ZSM (SNSB-Zoologische Staatssammlung, München, Germany, temporarily stored for further morphological work). TL – total length of beetle, TW – total width of beetle.

Morphological descriptions and photography

The description of morphological characters using an abbreviated written format supported by digital photographs of diagnostic structures as established in previous recent studies on similar taxa (Balke et al. 2020). Generic characters are not repeated in the species descriptions.

Images were taken with a Canon EOS R camera. We used Mitutoyo 20x ELWD Plan Apo objective for genital structures and a Canon MPE65 macro lens for the habitus photographs. The Mitutoyo lens was attached to a Carl Zeiss Jena Sonnar 3.5/135 MC, used as a focus lens. Illumination was with three LED segments SN-1 from Stonemaster (https://www.stonemaster-online-shop.de). Image stacks were generated with the Stackmaster macro rail (Stonemaster), and images were then assembled with the computer software Helicon Focus 4.77TM on a Mac Pro workstation using a Radeon Pro W6800X MPX module.

DNA analysis

nextRAD procedures were explained in detail in our previous publication, which was focused on biogeographic patterns of *Philaccolilus* across New Guinea (Lam et al. 2018). We also used traditional Sanger sequencing (SGS), where we sequenced the 3' fragment from the mitochondrial cytochrome c oxidase subunit 1 (CO1) gene, explained in (Lam 2017). Here, we used the sequence analysis program Geneious version R11 to assess the divergence between the different groups of sequences as uncorrected p-distances.

To examine the distribution of mtDNA sequence diversity within and between taxa, haplotype networks were constructed using the TCS algorithm (Clement et al. 2002) implemented in the PopART software (Leigh and Bryant 2015). In the networks below, each colored circle represents a mitochondrial haplotype. The color represents geographic locality. The size of the circle is proportional to the number of individuals with the haplotype (i.e. the larger the circle, the more specimens show exactly the same DNA sequence). Multiple specimens from the same locality usually show some degree of intraspecific difference (this is often cited as low genetic divergence, e.g. less than 1 or 2%). That means, that the sequence of the targeted gene fragment can be slightly different, with few nucleotide substitutions. The networks show this by connecting haplotypes with lines. Hashmarks represent a single nucleotide polymorphism (SNP). If there is only one hashmark on a connector, that means the two connected sequence types only differ by one nucleotide substitution. The more hashmarks, the more divergent are two connected haplotypes. A black dot on a connector indicates a hypothetical haplotype that should exist to explain the nucleotide diversity in a specific sequence position.

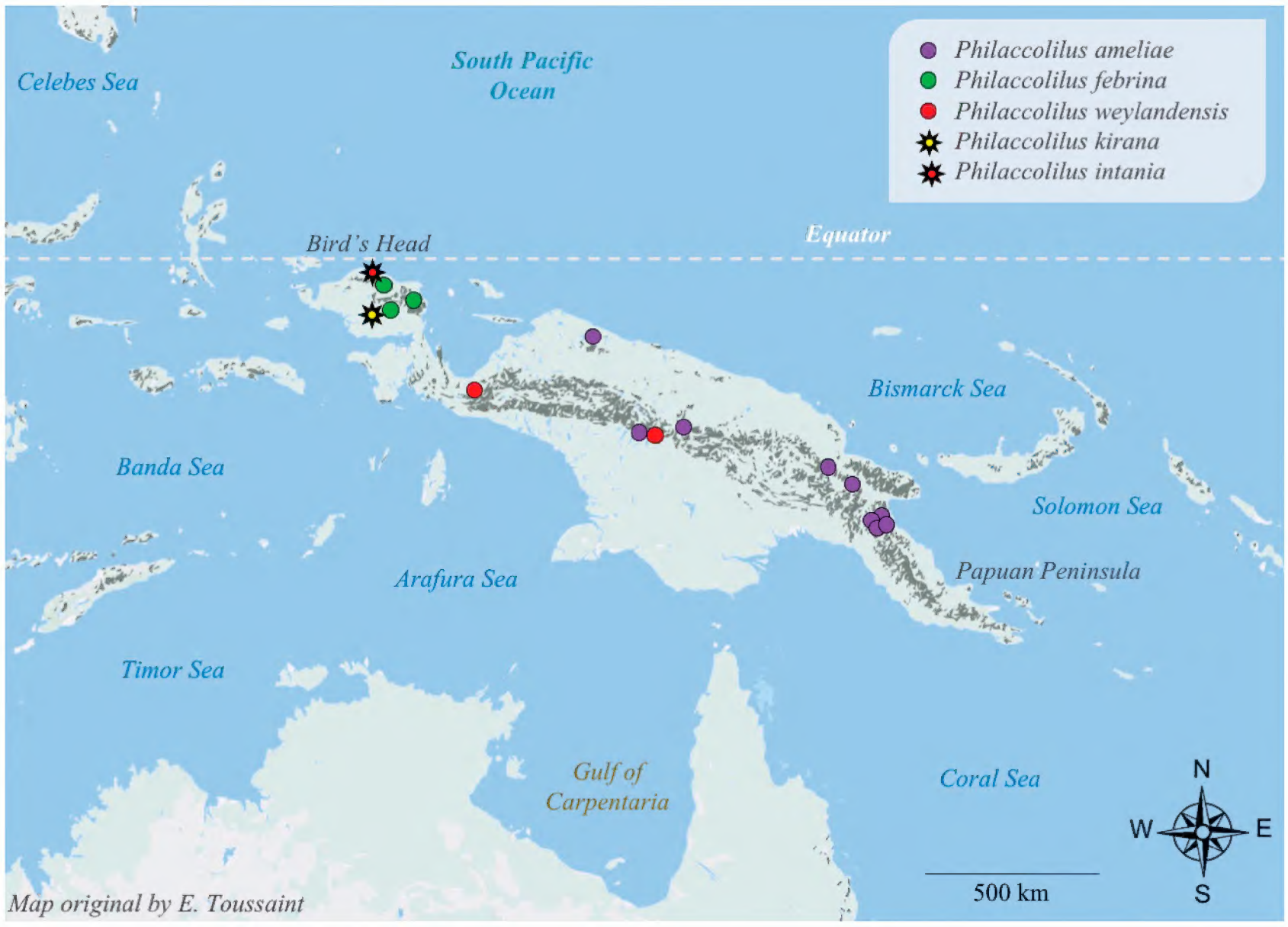

Figure 4. Distribution of *Philaccolilus* species in New Guinea. Dots – *P. ameliae* complex, stars – *P. kirana* and *P. intania* here described from the Bird's Head Peninsula.

To assess population structuring, we used a Bayesian clustering approach implemented in the program STRUCTURE 2.3.4 (Pritchard et al. 2000). We ran 10 replicates, each using a burn-in length of 100,000 and a run length of 1,000,000 steps, with the admixture and the correlated allele frequencies models without using prior population information (geographic sampling location). We tested number of clusters (K) from 1 to 18. The optimal number of distinct clades was determined by examining both the posterior probabilities of the data for each K and the ΔK estimator described by Evanno et al. (2005) as calculated in Structure Harvester (Earl and VonHoldt 2012). Results for the identified optimal values of K were summarized using CLUMPP ver. 1.1 (Jakobsson and Rosenberg 2007) using 1000 permutations and the LargeKGreedy algorithm; the result is then plotted using DISTRUCT ver. 1.1 (Rosenberg 2004).

In the STRUCTURE plots, each bar represents an individual and each block represents a geographic population. The colors of each bar represent their genetic population. An individual bar that contains multiple colors represents a sample with mixed ancestral population.

Results

Molecular phylogenetic evidence

Using nextRAD sequencing, we generated data from 1,726 genomic loci, from which we extracted 5,609 SNPs (single nucleotide polymorphisms) for population genomic analyses. For specimens tentatively identified as *P. ameliae* Balke et al. 2000, we recovered three well-delineated clades: one that contained 7 specimens from Sandaun Province and Waaf; one with 17 specimens from the Weyland Mts. and Brazza River, as well as one clade that contains 34 specimens from across the Bird's Head Peninsula (Fig. 5A, B). We concluded that what was referred to as one species, *P. ameliae* (with one subspecies, *P. ameliae weylandensis* Balke et al. 2000), should indeed be considered as three genetically well differentiated species. Specimens of *P. ameliae weylandensis* from the Weyland Mts. and Digul River formed one cluster, however with some degree of substructuring (Fig. 5B) (Lam et al. 2018). This indicates more hidden, genetic diversity. Specimens of *P. ameliae* from Waaf at the north coast and Sandaun (Mianmin) in the central highlands show a high degree of admixture, i.e. populations from these two far apart localities are not isolated genetically. We did not obtain nextRAD data for the population from Brazza River (Fig. 5B). The specimens from the Bird's Head Peninsula show a moderate degree of admixture (Fig. 5A), however with evident sign of substructuring between localities from the Arfak Mts. (Arfak, Testega) and the Tamrau Mts.

We also included 4 specimens of two populations of morphologically different putative species from the Bird's Head Peninsula for nextRAD sequencing (Lam 2017, as "P. band" and "P. black"). Population genomic results regarding species status were ambiguous, likely due to limited sampling (Figs 4, 5C). These could not be assigned to other known species from the main body of New Guinea island.

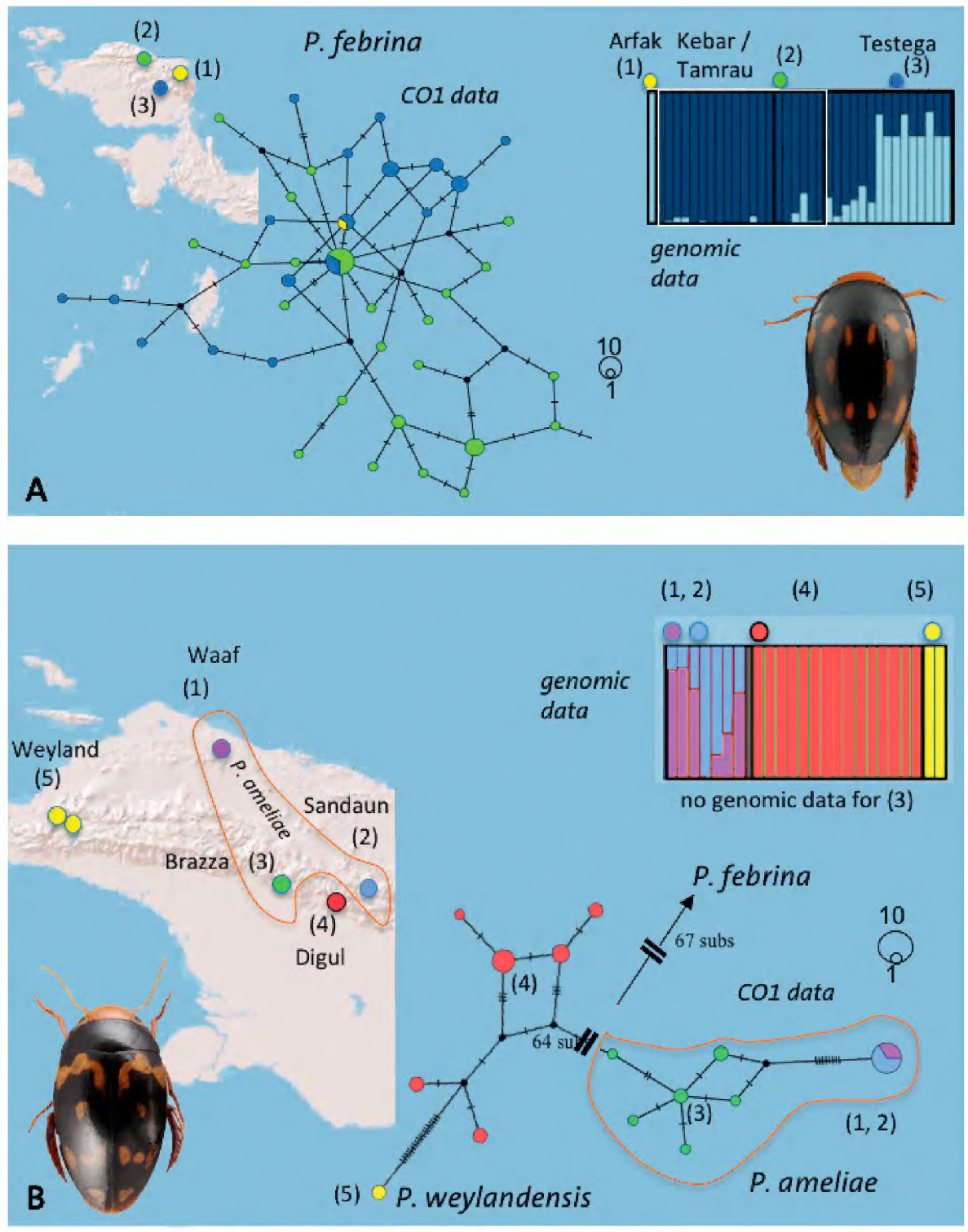

Figure 5. Results of the genomic nextRAD as well as mitochondrial DNA CO1 data sequencing of *Philaccolilus* species in New Guinea. **A**. *P. febrina* in the Bird's Head Pensinsula; **B**. *P. ameliae* and *P. weylandensis* in New Guinea main body; **C**. *P. kirana* and *P. intania* from the Bird's Head Peninsula (localities see **A**). Results of the structuring analysis of nextRAD data depicted as barplots, the CO1 data as haplotype network, subs.= substitutions.

All related statistical data were provided by Lam 2017 and Lam et al. 2018.

Sanger sequencing produced an alignment of 738 base pairs, for 98 specimens of *P. ameliae* as previously defined. We recover the same major clades as in the genome wide nextRAD data analysis. The CO1 divergence between these clades in comparably high, above 8% (Fig. 5B), with 64 substitutions between *P. ameliae weylandensis* and *P. ameliae* as well as 67 substitutions between these and the Bird's Head specimens. In *P. ameliae*, we find some degree of differentiation between the populations north of the central New Guinea watershed (Waaf, Sandaun) and the one from the south (Brazza River). The population of *P. ameliae weylandensis* from the Weyland Mts. and Digul River to the east are also moderately differentiated. These two are also from different sides of the central New Guinea watershed, north and south, respectively. In the CO1 haplotype network, the specimens from the Bird's Head Peninsula also show a moderate degree

of admixture (Fig. 5A), however with evident sign of substructuring between localities from the Arfak Mountains (Arfak, Testega) and the Tamrau Mts., as inferred from the nextRAD genomic data.

We also obtained data for 12 specimens of two populations of the above mentioned morphologically different putative species from the Bird's Head Peninsula (Fig. 5C), and find two clusters according to geographic locality (Testega in southern Arfak Mts. and the Tamrau Mts. to the north). The CO1 divergence between these is however very low, c. 0.4%, shown in the haplotype network in Fig. 5C.

Taxonomic implications based on these data are discussed and illustrated below.

Taxonomy

Genus *Philaccolilus* Guignot, 1937

Diagnosis. Laccophilinae with simple (pointed) metatibial spurs, simple lanceolate prosternal process (not trifid) (Fig. 8E), posteromedial pronotal margin more or less straight, elytral microreticulation with small transverse cells, metacoxal lines slightly diverging anteriorly (Fig. 8E), metatarsal lobes relatively long (hind tarsi therefore appearing broad, oar like) (Fig. 8E), without metacoxal/metafemoral stridulation device (Miller and Bergsten 2016) (Fig. 8E). Dorsal surface dark, with contrasting yellow / orange pattern, or completely black. The dorsal color pattern is usually delineating species (except for *P. irianensis* and *P. incognitus*, Fig. 1F, G). The extend of the paler markings can vary within species (e.g. Fig. 1J, K) and even left/right side in an individual, but not so the principal position and overall shape of the pattern. Therefore, the examination of multiple specimens is recommended. Penultimate ventrite with tuft of long golden setae medially. The median lobe of aedeagus is of, generally speaking, simple structure, i.e. simply curved sclerotized structure (e.g. Figs 6, 7).

Taxonomic decisions based on multiple data sources. The two lineages from different parts of the Bird's Head, "P. band" and "P. black" (Lam 2017) are genetically very similar. Based on CO1 data, the Arfak and Tamrau Mts. populations (Figs 4, 5C) are geographically structured, but only based on a low CO1 divergence. Based on that, their geographic distribution, clearly different body size and elytral color pattern, we suggest that they represent two species that evolved only recently in the two different mountain areas of the Bird's Head, after their ancestral species colonized that area out of the main body of New Guinea island.

What was so far referred to as *Philaccolilus ameliae* is a complex of at least three species. While morphological differences are subtle, both nextRAD and CO1 data clearly delineate three clades that we here assign species status. *Philaccolilus ameliae* is known from eastern and central New Guinea. *Philaccolilus febrina* is described from the Bird's Head (Fig. 5A). *Philaccolilus weylandensis* (new status) was described as a subspecies of *P. ameliae* from the west of New Guinea (Weyland Mts.) and was later also found in the Digul River on the southern slopes of the central range, more in the center of New Guinea (Fig. 4). Populations from these two localities are genetically differentiated and might in fact represent two species. We leave them assigned to *Philaccolilus weylandensis*, expecting additional samples from along the southern slopes of the central range. It is interesting to note that the only locality between Digul River and Weyland Mts. revealed a population of *Philaccolilus ameliae* (Fig. 5B). This hints to more complex diversification and biogeographic patterns in this complex, that requires additional fieldwork.

Philaccolilus intania sp. nov.

https://zoobank.org/4909B236-E61D-477A-8B74-A7A4A2352E41

Figs 2B, C, 3B, 4, 5C, 6C, D, 8A, B, 10F

Type locality. Tamrau Mts., Kebar, Bird's Head Peninsula, West Papua.

Holotype. Male. Indonesia, West Papua, above Kebar, forest creek, 720 m, 7.v.2015, -0,7831, 133,0721, UNIPA team (BH060) (MZB).

Paratypes. (MZB, KSP, ZSM) 25 exs, same label data as holotype; 5 exs, Indonesia, West Papua, Tamrau Mts N of Kebar, forest stream, 750 m, 7.xi.2013, -0,7831, 133,0721, UNIPA team (BH033). Note. BH033 and BH060 are the same creek, sampled in different years and slightly different stream section (+/- 50 meters).

Description of holotype. Medium sized member of the genus. TL 4.8 mm; TW 2.7 mm.

Color. Body surface black except for orange head as well as narrow, dark orange subbasal elytral band that not reaching lateral margin (Figs 2B, C, 3B).

Structures. Hind margin of last ventrite emarginate in the middle (Fig. 8A).

Genitalia. Median lobe of aedeagus as in Fig. 6C, D.

Female. Hind margin of last ventrite in the middle projected ("dwarf hat shape)" (Fig. 8B).

Variation. Size variation of the paratypes is (N=12) TL 4.7–5.1 mm (av. 4.9 mm); TW 2.6–2.9 mm (av. 2.8 mm). Orange subbasal elytral band is dissolved into isolated dots in some specimens.

Etymology. Named after Sophia Intania Balke, daughter of first author. The species name is a noun in the nominative singular standing in apposition.

Comparative notes. Distinguished from the other *Philaccolilus* species based on the following combination of features: body size; pronotum black; whole head orange; elytron only with a narrow, dark orange subbasal band not reaching the lateral margin (or only isolated spots in the position of the band); shape of median lobe (except for *P. kirana*, Fig. 6A, B).

Figure 6. Median lobe of aedeagus of *Philaccolilus* species **A, B**. *P. kirana*; **C, D**. *P. intania.*

Distribution. So far only known from the Tamrau Mts. in the north of the Bird's Head Peninsula of New Guinea (Fig. 4).

Habitat. Collected from a shaded forest stream, seen swimming on the sandy and gravelly bottom, in the current where pools form behind large rocks or underneath shallow cascades (Fig. 10F). After about 50 meters, that stream fed into a larger river which is depicted in Fig. 9C. In that larger, more exposed river, we only found *P. febrina*.

Philaccolilus kirana sp. nov.

https://zoobank.org/44488B73-3D65-43A6-948C-E868191EA71F

Figs 2A, 3A, 4, 5C, 6A, B, 8A, B, 10A–D

Type locality. Arfak Mts., Testega, Bird's Head Peninsula, West Papua.

Holotype. Male. Indonesia, West Papua, Testega, 1,210 m, 3.v.2015, -1,3686, 133,5908, UNIPA team (BH054) (MZB).

Paratypes. (MZB, KSP, ZSM) 38 exs, same label data as holotype; 8 exs, Indonesia, West Papua, Testega, 1,100 m, 1.v.2015, -1,3827, 133,5967, UNIPA team (BH052).

Description of holotype. Larger member of the genus. TL 5.6 mm; TW 3.1 mm.

Color. Body surface black except for orange head; anterior angle of pronotum very dark orange; elytron with broad, dark yellow subbasal band that reaching lateral margin; with small apical spot (Figs 2A, 3A).

Structures. Hind margin of last ventrite emarginate in the middle (Fig. 8A).

Genitalia. Median lobe of aedeagus as in Fig. 6A, B.

Female. Hind margin of last ventrite in the middle projected ("dwarf hat shape)" (Fig. 8B).

Variation. Size variation of the paratypes is (N=27) TL 5.0–5.6 mm (av. 5.3 mm); TW 2.9–3.2 mm (av. 3.0 mm). The subbasal elytral band is more or less constantly developed; apical spot is not evident in some specimens. One specimen has the apical portion of the right elytron paler, orange, and there are two small orange postmedial spots on the left elytron.

Etymology. Named after Maruscha Kirana Balke, daughter of first author. The species name is a noun in the nominative singular standing in apposition.

Comparative notes. Distinguished from the other *Philaccolilus* species based on the following combination of features: body size; pronotum black; whole head orange; elytron usually with only a broad, dark yellow subbasal band reaching the lateral margin and usually small apical spot; shape of median lobe (except for *P. intania* Fig. 6C, D).

Distribution. So far only known from the type locality and nearby, situated in the southern Arfak Mts. of the Bird's Head Peninsula of New Guinea (Fig. 4).

Habitat. Collected from a small lower order stream hidden in dense montane forest (Fig. 10D). Few specimens collected from a more or less sun exposed stream (Fig. 10A–C), together with *P. febrina*.

Figure 7. Median lobe of aedeagus of *Philaccolilus* species (left, lateral, right, ventral view) **A, B**. *P. ameliae* (from Brazza River); **C, D**. *P. ameliae* (paratype from Wau); **E, F**. *P. febrina* (from Tamrau Mts.); **G, H.** *P. weylandensis* (from Weyland Mts.); **I, J**. *P. weylandensis* (from Digul River).

Definition of the *Philaccolilus ameliae* complex

Philaccolilus ameliae Balke et al. (2000) was described from Morobe and Madang provinces in the eastern part of Papua New Guinea (Fig. 4), while the subspecies *Philaccolilus ameliae weylandensis* Balke et al. (2000) from much further west on the island, in the Weyland Mts. of Papua (Fig. 4). The subspecies was suggested based on the slightly narrower curvature of the median lobe in ventral view in *Philaccolilus a. weylandensis* (Balke et al. 2000). We find that the shape of the median lobe shows slight variation among specimens and possibly populations

(Fig. 7A–D, G–J), and differences are indeed subtle. The discovery of *Philaccolilus ameliae*-like specimens on the Bird's Head Peninsula and different areas in central New Guinea prompted an integrative taxonomic investigation of what we refer to as the *Philaccolilus ameliae* complex. We diagnose the complex as follows: moderately to larger sized beetles in the genus; pronotum black; whole head orange; elytral pattern dark yellow: narrow subbasal band with characteristic shape as depicted in Fig. 2D–H (basically three spots that can be isolated or more or less fused); a medio-discal dot or extended into longitudinal spot (in few specimens of *P. ameliae* extended posteriorly and reaching the postmedial band); postmedial band (of three isolated spots, rarely fused); an apical spot. The elytral color pattern is therefore variable, but constantly of the same general pattern and configuration (Fig. 2G). The outer spot of subbasal band can reach the lateral elytral margin (Fig. 3C), or hardly so (Fig. 3D) or not at all (Fig. 3E). Hind margin of male last ventrite truncate (Fig. 8C), in the female ("dwarf hat shape)" (Fig. 8D).

Philaccolilus ameliae Balke, Larson, Hendrich & Konyorah, 2000

Figs 2E, 4, 5B, 7A–D, 8C, D

Philaccolilus ameliae Balke, Larson, Hendrich & Konyorah, 2000: 35.

Type locality. Gusap, Markham Valley, Morobe Province, Papua New Guinea.

New material studied. (KSP, MZB, NARI, ZSM). 7 exs, PNG, Morobe, Herzog Mts., Patep, 700 m, 20.xi.2006, -6.9711, 146.6315, Balke & Kinibel (PNG 105); 14 exs, PNG, Sandaun, Mianmin, 1,000 m, 20.x.2008, -4.8881, 141.5686, Ibalim (PNG191); 2 exs, PNG, Sandaun, Mianmin (river), 700 m, 21.x.2008, -4.8809, 141.5284, Ibalim (PNG197); 14 exs, PNG, Sandaun, Mianmin area, 800 m, 6.i.2010, -4.9092, 141.6159, Ibalim & Pius (PNG239); Papua, Sarmi, Waaf, N Foja Mts, riverbank, 120 m, 23.ix.2014, -2,3445, 138,7395, Balke & Menufandu (Pap030); 9 exs, Papua, Dekai, upper Brazza, 273 m, 2./3.vi.2015, -4,7410, 139.6542, Sumoked (Pap044).

Note. All of these specimens from central New Guinea were assigned to *P. ameliae* based on matching them with a short CO1 sequence with four individuals from Morobe: Herzog Mts., Patep, which is part of the type area of *P. ameliae*. For the 300 basepair fragment obtained, the identity with Sandaun and Waaf specimens matched 99%. The short fragment for the Patep specimens was not used for the haplotype network in Fig. 5.

Description. Same as for the species complex. Moderately to larger sized member of the genus: the specimens from the Waaf population are on average (5.1 mm) smaller than from PNG localities (5.5 mm). The lateral spot of subbasal band not or at most hardly so in contact with lateral margin. The extend of the elytral spots is variable (Fig. 2H), the most extended configuration of the paler spots is only observed in few specimens and sometimes even only on one elytron. The outer spot of the subbasal band does not reach the elytral margin, or at most, in few specimens, vaguely so (as in Fig. 3D, E) (Table 1).

The medio-discal spot is typically narrow and longish, sometimes connected to the postmedial band.

Size. Paratypes from PNG: Wau TL (N=15) 5.3–5.7 mm (av. 5.5 mm), TW 2.9–3.1 mm; specimens from PNG: Mianmin (N=15) 5.3–5.9 mm (av. 5.5 mm), TW 2.9–3.1 mm; specimens from Papua: Waaf (N=9) 4.9–5.3 mm (av. 5.1 mm), TW 2.9–3.0 mm.

Genitalia. Median lobe of aedeagus as in Fig. 7A–D.

Distribution. Widespread from central to east New Guinea (Fig. 4).

Habitat. Collected from different stream types, but usually more sun exposed.

Philaccolilus weylandensis Balke, Larson, Hendrich & Konyorah, 2000, stat. nov.

Figs 2F, G, 3C, D, 4, 5B, 7G–J, 8C, D

Philaccolilus ameliae weylandensis Balke, Larson, Hendrich & Konyorah, 2000: 35.

Type locality. Weyland Mts., southern Nabire, Papua.

New material studied. (KSP, MZB, ZSM) 49 exs, S Ok Sibil, tributary of Digul Riv, 292 m, 7./9.vi.2015, -5.0917, 140.7087, Sumoked (Pap046); 7 exs, Papua, N Waaf vill, pondok, 150m, 4.–7.vi.2016, -2.4061, 138.7439, Sumoked (Pap061).

Description. Same as for the species complex. Larger sized member of the genus. The lateral spot of subbasal band is broadly in contact with lateral margin in all specimens from the Weyland Mts. and in some from the Digul River. Habitus and color pattern are as in Fig. 2F, G. The outer spot of the subbasal band of Weyland Mts. specimens does reach the elytral margin, or, in few specimens, vaguely so (as in Fig. 3C, D) (Table 1). In the Digul River specimens (Fig. 3D), in most specimens the subbasal band only vaguely reaches the elytral margin (the outer dot is rarely isolated and rarely fully in touch with margin (Table 1)). As such, generally speaking, *Philaccolilus weylandensis* is characterized by a trend to possess this out spot being more or less in contact with the elytal margin.

Table 1. Color variation on elytra in *P. ameliae* species complex.

	Outer basal lateral spot reaching elytral margin?		
	no	**vaguely**	**yes**
P. ameliae			
Wau	x	(x)	
Sandaun	x		
Foja	x		
Brazza	x	(x)	
Patep	x		
P. weylandensis			
Weyland Mts.		(x)	x
Digul River	(x)	x	(x)
P. febrina	x		

Figure 8. Last male and female ventrite of *Philaccolilus* **A, B**. As in *P. kirana* and *P. intania*; **C, D**. As in *P. ameliae*, *P. febrina* and *P. weylandensis*. Not to scale. In **C.** with last and penultimate ventrites to illustrate the tuft of long golden setae on penultimate ventrite; **E**. *P. febrina* male in ventral view, not to scale.

The medio-discal spot is typically shorter and somewhat broader, or even only a dot (Figs 2F, G) and never fused with postmedial band.

Size. Paratypes from the Weyland Mts. TL (N=6) 5.5–5.7 mm (av. 5.6 mm), TW 3.0–3.1 mm; specimens from Ok Sibil (N=12) 5.3–5.6 mm (av. 5.4 mm), TW 3.0–3.1 mm.

Genitalia. Median lobe of aedeagus as in Fig. 7I, J.

Distribution. Weyland Mts., localities north of the central watershed; as well as Digul River south of Ok Sibil, which is south of the central watershed (Fig. 4).

Habitat. Collected from different stream types, but usually more sun exposed.

***Philaccolilus febrina* sp. nov.**

https://zoobank.org/C1C8B146-9242-4CB6-AAEC-15ED651AC272

Figs 2D, as in G in part; 4, 5A, 7E, F, 8C, D, 9A–D, 10A–C, E–F

Type locality. Tamrau Mts., Kebar, Bird's Head Peninsula, West Papua.

Holotype. Male. Indonesia, West Papua, Tamrau Mts. N of Kebar, forest stream, 750 m, 7.xi.2013, -0.7831, 133.0721, UNIPA team (BH033) (MZB).

Paratypes. (MZB, KSP, ZSM) 4 exs, same label data as holotype; 26 exs, Indonesia, West Papua, Road Manokwari – Mokwam, 320 m, 25./27.i.2006, 01 00.596S 133 53.921E,

Figure 9. A–D. Habitats of *Philaccolilus febrina*. **A** Stream more or less on level of valley floor, locality BH62; **B–D.** Locality BH61, open stream flowing out of Tamrau Mts. and feeding into the stream shown in Fig. 9A.

Tindige, Prativi & Balke (BH01) ; 5 exs, West Papua, Indabre River, 1,300 m, 8.iv.2007, -1.1122, 133.8745, Sites & Supuma; 1 ex., Testega, ca. 1,100 m, 1.v.2015, -1.3829, 133.5992, UNIPA team; 36 exs, West Papua, Testega, 1,100 m, 1.v.2015, -1.3827, 133.5967, UNIPA team (BH052); 36 exs, West Papua, above Kebar, forest creek, 720 m, 7.v.2015, -0.7831, 133.0721, UNIPA team (BH060); 112 exs, West Papua, above Kebar, open forest stream, 720 m, 7.v.2015, -0.7856, 133.0712, UNIPA team (BH061); 93 exs, West Papua, above Kebar, open river, 600 m, 7.v.2015, -0.8009, 133.0578, UNIPA team (BH062) (note: mislabelled as BH061); 63 exs, West Papua, Kebar Valley, 600 m, 8.v.2015, -0.8348, 133.1839, UNIPA team (BH063).

Description of holotype. Moderately to larger sized member of the genus. Total length of beetle: 5.0 mm; maximum width: 2.7 mm.

Color. Body surface black except for dark yellow head; anterior half of pronotum lateral margins dark orange; elytron with dark yellow subbasal "band" composed of three spots, hardly fused, not reaching the lateral margin (as in Fig. 3E); with medio-discal spot; with postmedial "band" composed of three spots; with small apical spot (Fig. 2D).

Structures. Hind margin of last ventrite truncate (Fig. 8C).

Genitalia. Median lobe of aedeagus as in Fig. 5E, F.

Female. Hind margin of last ventrite in the middle projected ("dwarf hat shape)" (Fig. 8D).

Variation. Size variation of the paratypes is (N=25) TL 4.7–5.3 mm (av. 5.0 mm); TW 2.5–2.8 mm (av. 2.7 mm). The elytral spots can be more or less elongated, rarely some of them fused; medio-discal spot and postmedial "band" are not in touch (only in one specimen and only on one elytron almost as in Fig. 2H, right) in any of the studied specimens of *Philaccolilus febrina*.

Etymology. Named after Ditta Febrina Amran Balke, wife of first author and ZSM Coleoptera collection and project manager. The species name is a noun in the nominative singular standing in apposition.

Comparative notes. Distinguished from the other *Philaccolilus* species based on the following combination of features: body size; pronotum black; whole head orange; characteristic general dark yellow pattern on elytron (Fig. 2D, G); shape of median lobe (Fig. 7E, F). Very similar to *P. ameliae* and *P. weylandensis*. Morphologically, *Philaccolilus febrina* differs from the previous species of the *P. ameliae* complex by slightly smaller average size and the slightly broader median lobe in ventral view (Fig. 7F).

Distribution. Widespread in the Tamrau and Arfak mountains (Fig. 4).

Habitat. Collected from different stream types, but usually more sun exposed (Figs 9A–D, 10A–C, E). Also found in a forest stream, seen swimming at the sandy and gravelly bottom, of a larger pool behind large rocks (Fig. 10F).

Figure 10. Habitats of *Philaccolilus* species **A–C.** *P. kirana* and *P. febrina* locality BH052; **D.** *P. kirana* BH54; **E**. *P. febrina* BH63; **F**. *P. intania* and *P. febrina* BH60.

Conclusions

Combining morphological examination, traditional single-gene fragment sequencing and population genomic analyses, we improve our understanding of New Guinea stream beetles in the genus *Philaccolilus*. We previously uncovered cryptic diversity among populations in the *P. ameliae* complex, with complex patterns of genetic connectivity (Lam et al. 2018), where for example remote populations of *P. ameliae* from Sandaun and Waaf (Fig. 5B) share the same mitochondrial haplotype, while closer Sandaun and Brazza populations are well differentiated, but divided by the central watershed. We suggest that much more comprehensive field sampling will be required to untangle the biotic and abiotic factors leading to such patterns. Large scale biogeographic investigations of the New Guinea biota are currently becoming more abundant, and it is desirable to design population genomic studies to provide more data on the speciation processes behind them.

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